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Some Causes of Genotypic and Phenotypic Discordance in Monozygotic Twin Pairs

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The use of the adjective "identical" rather than monozygotic leads to misunderstandings about the biology of monozygotic twinning. Most monozygotic twin pairs are not identical; there may be major discordance for birth weight, genetic disease, and congenital anomalies. These indicate that post-zygotic events may lead to the formation of two or more cell clones in the inner cell mass and early embryo that actually stimulate the monozygotic twinning event. There is also evidence that there may be unequal allocation of numbers of cells to the monozygotic twins; this may have widespread implications for the cascade of developmental events during embryogenesis, formation, and vascularization of the placenta.

Large-scale zygosity testing at birth could be the template for analysis of twin outcomes and their biologic causes.

KEY WORDS: diseases in twins, monozygotic twins, embryogenesis

INTRODUCTION

Monozygotic (MZ) twins continue to be analyzed for the prevalence and cause of multifactorial diseases. Such studies are based on the premises that MZ twin pairs are genetically "identical," but were exposed to different postnatal environments. For instance, cohorts of MZ twin pairs are monitored for non-genetic influences on risks for developing diabetes and cardiovascular disease [Newman et al., 1990]. The assumption of such studies is that ". . . by comparing within genetically identical MZ twin pairs, they have eliminated genetic variability, thereby creating a pure culture of non-genetic determinants—determinants they define as being 'environmental exposure' and 'personal behaviors' . . ." [Van Itallie and Stunkard, 1990]. However, the work of Bouchard et al. [1990] clearly indicates that "personal behaviors" of MZ twins reared apart are surprisingly similar. Attempts to dissect out genetic and environmental factors in skin nevus counts and their progression to melanoma are similarly flawed. There is strong correlation between nevus counts within MZ twin pairs, but not in dizygotic (DZ) twin pairs [Easton et al., 1992]. However, attempts to distinguish between genetic predisposition to nevi and the effects of sun exposure cannot be made, since a tendency to sun exposure might be genetically determined in MZ twins [Cantor, 1992].

While it is true that most of MZ twins are phenotypically very similar, there are significant numbers of MZ pairs who are neither genotypically nor phenotypically "identical." Post-zygotic genetic events of various types and severity result in striking within-pair dissimilarity; indeed, it is proposed that, in some pairs, these post-zygotic events not only precede the twinning process, but may actually trigger it; if two recognizably different cell clones exist in one early zygote, the differences may be sufficient to cause mutual "recognition and repulsion," with resulting MZ twinning. Such dissimilarities strike at the heart of twin studies, since they point to genetic dissimilarity in some, and perhaps many, MZ twin pairs.

Even if there are also extrinsic events that cause MZ twinning (such as traumatic rupture of the zona pellucida during hatching), an important characteristic is that the allocation of blastomeres to the two embryos may not be equal. Hence, the development of the twins may run non-parallel courses from the moment of the twinning event. Certain critical masses of cells may be necessary for the cascade of normal transcriptional events that leads to cell lineages and differentiation following gastrulation. It is easy to see how "vanishing twins," fetus papyraceus, and holoacardius acephalus (twin reversed arterial perfusion) might result from such unequal allocations of cell populations. In the case of X-chromosome inactivation in female MZ twin pairs, there is good evidence for unequal allocation of blastomeres in some cases (see below).

It should also be noted that, for each MZ twin to approach DZ twin or singleton birth weight for a given

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gestational age, the whole zygote needs to pass through one extra mitotic cycle. The implications for this on timing and differentiation are not clear. If there is unequal allocation of blastomeres, the smaller twin might need more than one extra mitotic cycle to achieve normal body weight.

A further point is that the formation of MZ twins must result in major reorientation of at least one of the major axes of the body plan. Given that higher vertebrates undergo axial orientation and regional specification largely by inductive (or regulative) cell-to-cell interactions whose effects begin after gastrulation, it seems likely that the first of the three axes (possibly dorso-ventral) [Bocklage, 1981, 1987] is laid down in the oocyte cytoplasm as a maternal effect, i.e., by regionally unequal topographic distribution of maternal mRNAs that specify the position of polar trophoblast; thereafter, the cranio-caudal axis of gastrulation is specified early, and the third axis, left-right, is probably specified inductively somewhat later, although the molecular parameters may be laid out before gastrulation. In MZ twinning, it is easy to see that cranio-caudal and dorso-ventral axes (or gradients) could be transmitted fairly faithfully from a single zygote to the two gastrulation processes; but left-right asymmetry might well be disturbed. This could account for some cases of abnormal heart looping, situs inversus/ambiguus/heterotaxy and proposed mirror-imaging in MZ twins.

By corollary, it would follow that all animals with a virtually autonomous (i.e., mosaic) regional specification of body regions would be unable to undergo MZ twinning, since this would result in reciprocal deficiencies in the body plans of the twins (e.g., one largely dorsal or animal, the other largely ventral or vegetal, etc.).

A further important factor is that two-thirds of MZ twins share a truly single, monochorionic placenta, with the potential for asymmetrical unequal sharing of venous return from the placental parenchyma. Thus, even the prenatal environment for monochorionic MZ twin pairs may be more dissimilar than that for dichorionic twins (see accompanying paper).

The purpose of this paper is to draw attention to the several known and hypothesized factors that may cause genotypic and phenotypic dissimilarity in MZ twin pairs. The facile use of MZ and DZ twins to distinguish "genetic" and "environmental" influences on development and disease is to be discouraged [Phillips, 1993]. Thorough documentation of zygosity and chorionicity by prenatal and perinatal diagnosis [Derom et al., 1991] allows certain parameters of similar and dissimilar development to be measured in MZ twins (*see accompanying paper* [Machin et al., 1995]). Careful study of MZ pairs who are dissimilar will yield important information on the likely causes and effects of MZ twinning.

SOME CLUES TO THE ETIOLOGY OF MZ TWINNING

The timing of the MZ twinning events has been linked to placentation. One-third of MZ twins have dichorionic placentas, separate or fused. This implies an early separation of two groups of blastomeres, with each twin subsequently developing its own trophoblast and inner cell mass. Two-thirds of MZ twins are monochorionic; in these twins, the "decision" for twinning is made later and is limited to the inner cell mass after it has separated from the trophoblast. There is discordance between the number of inner cell masses and the number of trophoblasts. A small proportion of monochorionic MZ twins are also monoamniotic, indicating an even later twinning event, when the amnion has become distinct from the inner cell mass(es). Most conjoined twins are monochorionic monoamniotic. They have two body axes in one body, implying a very late and variably incomplete attempt at MZ twinning.

Thus, the four MZ types, identified by their placentation as being of different timing, could be caused by different events. The only major pointer is a steadily rising female excess from dichorionic to conjoined MZ twins (Fig. 1) [Derom et al., 1988; James, 1980; Gedda et al., 1981]. It seems likely that dichorionic MZ twins are fundamentally different from monochorionic MZ twins in their origin and biology, apart from the fact that they are spared the disadvantages of a monochorionic placenta. Monochorionic MZ twins are significantly at risk for non-genetic prenatal environmental influences that distinguish them from dichorionic MZ twins.

Potentially informative placental arrangements are to be found in MZ higher multiple gestations. Here there may be combinations of dichorionic and monochorionic placentations, indicating serial twinning

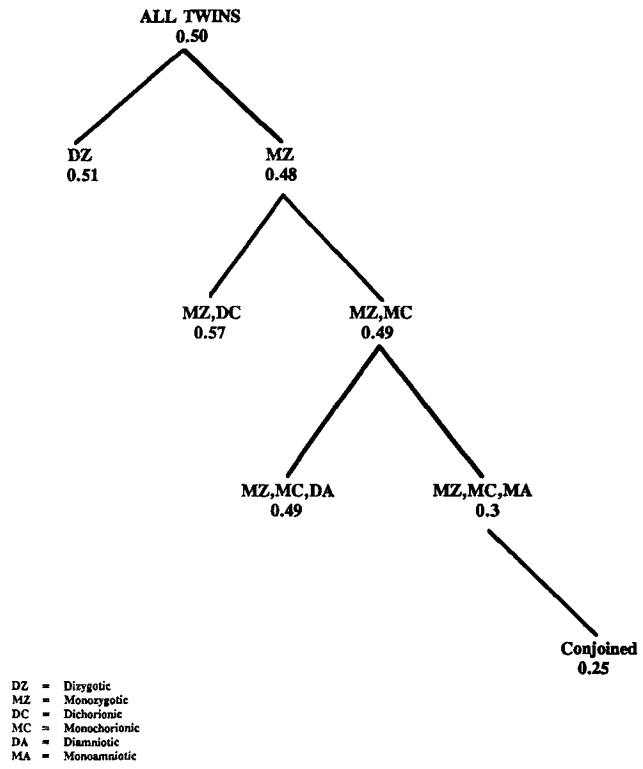

Fig. 1. Sex ratios in twins by zygosity, chorion, and amnion status. There is a steadily increasing female excess in MZ twins arising from later twinning events [based on data by Derom et al., 1988; James, 1980; Gedda et al., 1981].

events that have happened at different times after conception. Significant similarities and differences between monochorionic and dichorionic twins among MZ triplets or quadruplets could point to the timing and effects of the post-zygotic triggers of twinning (*see accompanying paper*) (Fig. 2) [Machin and Bamforth, 1996].

POST-ZYGOTIC GENETIC EVENTS

Two major types of post-zygotic genetic events are now known to distinguish some MZ twin pairs (although chorionicity is not always documented):

1. Chromosomal Mosaicism in MZ Twins

The situation is complicated by three factors, viz., the timing of the event (even into somatic regions of one twin), the placental status of the chromosomes (i.e., interacting effects of confined placental mosaicism and confined MZ twin mosaicism), and the presence or absence of interfetal anastomoses, which can superimpose blood chimerism, even among MZ twins.

MZ twins have been reported concordant for all four of the common human aneuploidies; 45,X [Reiss et al., 1993; Heydanus et al., 1993, case 22; Lin and Garver, 1988], 21-trisomy [Shapiro and Farnsworth, 1972; Rogers et al., 1982; Zellweger, 1968], 18-trisomy [Mulder et al., 1989; Schlessel et al., 1990], and 13-trisomy [Loevy et al., 1985]. In many of these pairs, there is discordance for the phenotypic expression of the aneuploidy, although mosaicism is not invoked. However, for 45,X,21-trisomy and 13-trisomy, mosaicisms have been reported in MZ twin pairs to account for striking phenotypic discordance. Mosaicism is common among X-chromosome aneuploid singletons (some of whom may be sole survivors of MZ twin pairs). In MZ twin pairs discordant for 45,X descent from 47,XXY, 47,XXX, 46,XY, and 46,XX zygotes has been reported (Table I). In many of these cases, fibroblast cell lines show complete dichotomy of the two cell lines between the embryos, and the presence of two cell lines in blood of one or both twins is presumed to be caused by MZ blood chimerism via vascular anastomoses in monochorionic placentas. Others have mosaicism in fixed tissues also. In some informative cases, non-disjunction events appear to have taken place at first post-zygotic division. Kurosawa et al. [1992] reported MZ twins with 47,XXY and 45,X karyotypes, without any evidence of a 46,XY cell line. Ross et al. [1969] reported on a pair of MZ twins with 47,XXX and 45,X cell lines without any residual 46,XX cells.

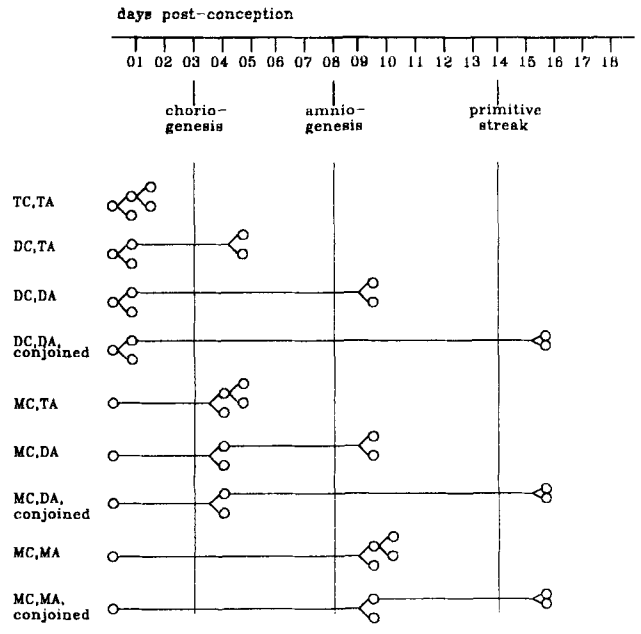

Fig. 2. MZ higher multiple gestations may show evidence of multiple twinning events occurring in time clusters or occurring successively over intervals of up to several days.

The following types of twin mosaicism could arise from a single 46,XY zygote by single post-zygotic non-disjunctional events: 46,XY and 45,X; 46,XY and 46,XY/45,X; 46,XY/45,X and 45,X; 46,XY/45,X and 46,XY/45,X; 47,XXY and 45,X; 47,XXY and 47,XXY/45,X; 47,XXY/45,X and 45,X; 47,XXY/45,X and 47,XXY/45,X; as well as mosaic combinations of the three cell lines, 47,XXY/46,XY/45,X. Similar considerations apply to 46,XX/45,X, 47,XXY/45,X, and 47,XXX/45,X mosaicism. In theory, two different non-disjunctional events could lead to MZ 46,XY and 46,XX embryos derived from a single 47,XXY zygote. X-chromosome non-disjunctions have been reported in MZ triplets [Dallapicolla et al., 1985; Landy et al., 1988]. The triplets reported by Dallapicolla et al. [1985] were MC, implying a relatively late post-zygotic non-disjunction.

45,X chromosome status can confer hemizygosity for X-linked disease, and symptomatic Duchenne muscular dystrophy may occur in singleton X-chromosome aneuploid mosaic patients [Bortolini et al., 1986]. Bonilla et al. [1990] reported on MZ twin sisters, one of whom had clinical and laboratory evidence of Duchenne muscular dystrophy. The asymptomatic sister had an affected son. 45,X/46,XX mosaicism was present in lymphocytes but not fibroblasts of the affected twin. The unaffected twin had 46,XX chromosomes in lymphocytes.

MZ twins discordant for trisomy 21 have been documented. Rogers et al. [1982] reported monochorionic male twins who were discordant for trisomy 21. Each was non-mosaic in fibroblast lines, but there was 46,XY/47,XY,1 21 chimerism in blood. Shapiro and Farnsworth [1972] described discordant females who were MZ by multiple blood group antigen testing. One twin was 46,XX and the other was a 46,XX/47,XX,1 21 mosaic (or chimera). Both twins had atrioventricularis communis.

One pair of monochorionic MZ male twins has been reported as discordant for trisomy-13 [Heydanus et al., 1993, case 2].

In all cases with an originally trisomic zygote, considerations of uniparental heterodisomy apply to the euploid twins.

MZ twin mosaicism has also been found for structural chromosome abnormalities. Fujimoto et al. [1991] described twins who were MZ by multiple antigen testing to a probability of 0.9977. The twins were both ab-

TABLE I. X-Chromosome Aneuploidy Mosaicism*

Author	Twin A fibroblasts	Twin B fibroblasts	Blood chimerism	MZ testing
Edwards et al., 1966	UT 45,X	NM 45,X	45,X/46,XY	
Karp et al., 1975	UT 45,X/46,XY	NM 2	45,X/46,XY	BGA
Schmidt et al., 1976	UT 45,X	NM 46,XY	None	BGA
Reindollar et al., 1987	UT 45,X/46,XY	NM 45,X/46,XY	?	MC
Arizawa et al., 1988	UT 45,X	NM 46,XY	None	
Turpin et al., 1961	UT 45,X	NM 46,XY	2	MC
Perlman et al., 1990	UT 45,X	NM 46,XY	?	MC
Wapner et al., 1990	UT 45,X	NM 46,XY	2	MC
Dallapicolla et al., 1985	UT 45,X	NM 3 2 (triplets) 46,XY		MC
Kurosawa et al., 1992	UT 45,X/47,XXY	45,X/47,XXY		RFLP
Deacon et al., 1980	TRAP, features UT 45,X	NF pump twin 46,XX	45,X/46,XX	MC
Pedersen et al., 1980	UT 45,X	NF 2		BGA-9932
Ross et al., 1969	UT 45,X/47,XXX	NF 45,X/47,XXX		BGA 70-99
Kaplowitz et al., 1991	UT 45,X	NF 46,XX	45,X/46,XX	RFLP
Neilson et al., 1982 (case 3)	UT 45,X	NF 46,XX		MC
Reiss et al., 1993	UT 45,X	NF 46,XX		RFLP

*NF, normal female phenotype; NM, normal male phenotype; UT, Ullrich-Turner phenotype; BGA, blood group antigens; RFLP, restrictive fragment length polymorphism.

normal, one having an Ullrich-Turner phenotype and a fibroblast chromosome constitution of 45,X. The cotwin was genitally male, and fibroblast chromosome analysis was 45,X (78%)/46,X,idic(Y)(p11) (22%). Both had blood chimerism. The structurally abnormal Y, present in the zygote, was presumably lost from a progenitor embryonic cell by anaphase lag. Watson et al. [1990] reported monochorionic MZ twins who were discordant for a Y/1 translocation with partial trisomy 1. Chromosomes from fibroblasts of the malformed twin were 46,XY/46,X,2 Y,1 der(Y), t(Y;1)(q12;q21). The cotwin had a 46,XY karyotype from lymphocytes. The mutational event was presumably post-zygotic. In a well-studied case, Lacassie et al. [1993] reported twins who were proven MZ by DNA mini-satellite analysis and who were discordant for a 13r chromosome. The normal twin was a 46,XX female by blood lymphocytes, as was the abnormal twin. However, the second twin had clear evidence of left lateralized somatic mosaicism, and skin fibroblasts from the left and right sides showed 13% and 2.6% of cells respectively with a 46,XX,2 13,1 13r karyotype. Tsukamoto et al. [1993] reported on MZ twins who were discordant for 7q2 syndrome. The phenotypically normal twin had a 46,XY karyotype, while the abnormal twin had a 46,XY,del(7)(q32→qter) chromosome constitution. There was lymphocyte chimerism. Juberg et al. [1981] reported monochorionic MZ twins discordant for 46,XY,del(10)(p11p15). It is notable that the placenta, although monochorionic, had two discs.

Several other cases of mosaicism for structural chromosome abnormalities in the context of twin reversed arterial perfusion are listed by Wolf et al. [1991].

The question of ploidy "mosaicism" (or chimerism) is complex. Both in singletons and twins, fertilization of a first polar body and of the ovum actually results in two zygotes, and hence, true chimerism, derived from two sperm but only one primary oocyte [Kennerknecht et al., 1991; Tuerlings et al., 1993; Bieber et al., 1981]. The twins reported by Bieber et al. [1981] were MC; a normal 46,XY male and a holoacardiac 69,XXX fetus shared the same placenta, which had the necessary inter-fetal anastomoses to support the acardiac fetus. Using HLA haplotypes, it could be shown that the triploid acardiac was heterodisomic for the maternal contribution; this was derived from a first polar body with evidence of crossing over, since both maternal haplotypes were represented on the chromosome of the polar body. Hence, these twins, although monochorionic, were actually DZ, with true chimerism rather than mosaicism. Wulfsberg et al. [1991] reported monochorionic female twins who probably had twin transfusion syndrome. One twin was phenotypically normal, with 46,XX chromosomes. The other twin had 46,XX/69,XXX mosaicism (65%/35%) in skin fibroblasts with clear evidence of asymmetry for dysmorphism, including cutaneous pigmentary dysplasia.

2. Skewed X-Chromosome Inactivation in MZ Female Twins

Non-random X-chromosome inactivation of structurally normal X-chromosomes is one possible cause for expression of X-linked disease in females [Wadeluis et al., 1993]. Skewed X-chromosome inactivation has also been proposed as the cause of discordant phenotypic expression of X-linked diseases in female MZ twin pairs. Several reports of such discordance are now well documented (Table II). The hypothesis depends on the fact that there have been no reported cases of female MZ twins, obligatory or proven heterozygotes for X-linked disease, in whom either both or neither are affected. In all cases, there is phenotypic discordance. However, see also Goodship et al. [1996]. There is good evidence that the clinically affected twin has non-random inactivation predominantly of the X-chromosome carrying the wild gene, while the unaffected twin either has predominant inactivation of the X-chromosome carrying the mutant gene or has random X-inactivation. Some pairs with skewed/random X-inactivation showed clear evidence of unequal allocation of blastomeres to the twin embryos.

TABLE II. Non-Random X-Inactivation in Female MZ Twin Pairs With Clinical Expression of X-Linked Diseases*

Author	Disease	Inactivation detection method	Affected twin DNA source		Normal twin DNA source		Possible mechanism	Pedigree
			F	L	F	L		
Tuckerman et al., 1985	XLMR	BRdU incorporation	2	Sk to wild	2	Sk to mutant	RSk, pre-T	
Burn et al., 1986	DMD	Somatic cell hybrids	Sk to wild	2	Sk to mutant	2	RSk, pre-T	? de novo, gonadal mosaic
Richards et al., 1990	DMD	DXS255	Sk to wild	Sk to wild	Sk to mutant	Sk to mutant	RSk, pre-T	maternal carrier
Lupski et al., 1991	DMD	DXS255	2	Sk to wild	2	R	UBA, post-T	? de novo, normal twin has affected boy
Abbadi et al., 1992	DMD	DXS255	2	Sk to wild	2	Sk to mutant	RSk, pre-T	mother, sister carriers; affected brother
Jorgensen et al., 1992	RGCB	DXS255	Sk to wild	R	Sk to mutant	R	UBA post-T, blood chimerism	?
Winchester et al., 1992	Hunter disease	DXS255 (M27b)	Sk to wild	Sk to wild	2	R	UBA, post-T	Maternal carrier
Kruyer et al., 1993	XLMR	DXS255 (M27b)	2	Sk to wild	2	Sk to mutant	RSk, pre-T	

*F, fibroblasts; L, leucocytes; XLMR, X-linked mental retardation; Sk, skewed; RSk, reciprocal skewed X-inactivation; pre-T, occurs before twinning; DMD, Duchenne muscular dystrophy; R, random; UBA, unequal blastomere allocation; post-T, occurs after twinning; RGCB, red-green color blindness. Placental chorionicity was not known in any of these cases.

In effect, there are two possible mechanisms (Fig. 3); in one, there is reciprocal skewed X-inactivation, which may result from the aggregation/segregation within the inner cell mass of two clones of cells with dissimilar X-inactivation status, followed by mutual repulsion from the oppositely inactivated clones. In the other mechanism, there may be random X-inactivation in the inner cell mass, but unequal allocation of blastomeres to the twins. In the twin who is allocated very few progenitor blastomeres, it is likely that, by chance, there will be skewed X-inactivation, while the co-twin, derived from a larger number of blastomeres, will show random or near random X-inactivation. In the first mechanism, X-inactivation would precede and (might trigger) MZ twinning; the second mechanism might be extrinsically imposed on the zygote after or independent of X-inactivation. This mechanism would result in unequal patch sizes in the twin pair [Nance, 1990; Zneimer et al., 1993]. Reported cases of non-random X-inactivation in female MZ twins are shown in Table II, according to method of DNA analysis and likely mechanism of distribution of X-inactivation.

An obvious question is whether the process of X-inactivation is normally skewed (reciprocally or otherwise) in MZ female twin pairs who are not heterozygous for a mutant X-linked genetic disease. If skewed X-inactivation is normally present, it could act as a trigger to the MZ twinning process, and account for the known excess of females among MZ twins, most marked among monochorionic MZ twin pairs. If unequal X-inactivation is not demonstrable in non-diseased female MZ twins, it would be necessary to suggest that the mutant gene itself is in some way the stimulus to non-random X-inactivation.

Another possibility is that apparently singleton females, with X-linked disease secondary to skewed X-inactivation [Wadelius et al., 1993], may actually be surviving MZ twins [Bundy, 1991]. The patterns and role of X chromosome inactivation in MZ twinning are further discussed by Trejo et al. [1994] and, in accompanying papers in this symposium, by Goodship et al. [1996] and Bamforth et al. [1996].

3. Post-Zygotic Dominant Gene Mutation

This author is not aware of any reports of proven post-zygotic mutations leading to discordance for autosomal or X-linked dominant single gene discordance in MZ twins. However, MZ twins may be discordant for severity of clinical expression of such diseases. Discordance for cloverleaf skull has been reported in three pairs of MZ twins with thanatophoric dysplasia [Horton et al., 1983; Serville et al., 1984; Young et al., 1989]. In a pair of MZ twins with neurofibromatosis (probably a de novo mutation), there was markedly different density of café-au-lait spots [Vaughn et al., 1981]. The twin with more numerous and larger café-au-lait spots developed an optic glioma at the age of two. The twins were of equal birth weight, but there is a suggestion here of discrepancy in the size of clones (or patches) of cells. In six pairs of affected MZ twins, Easton et al. [1993] found high concordance for density of café-au-lait spots and neurofibromata. Costa et al.

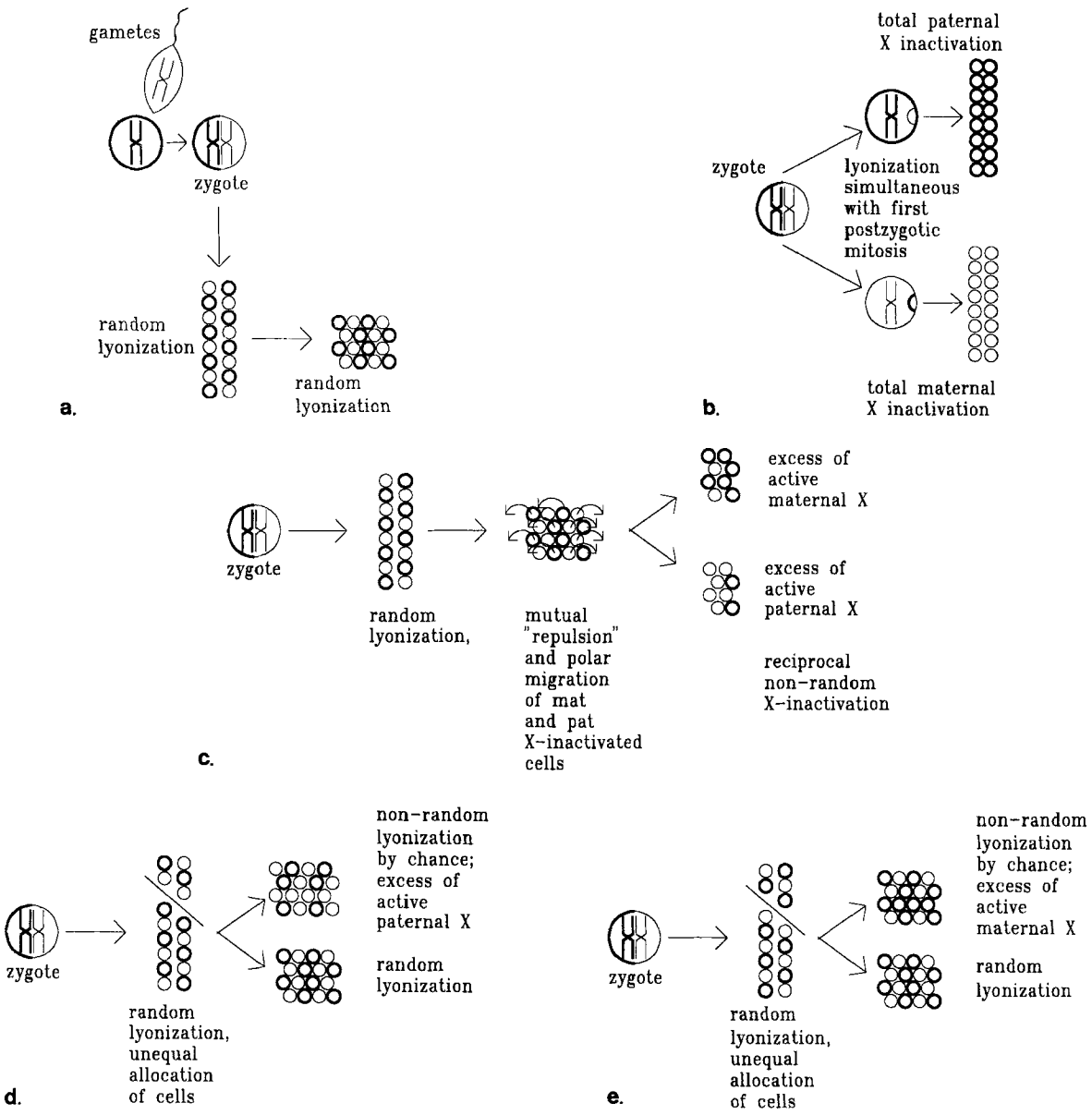

Fig. 3. Two possible mechanisms of non-random X-inactivation (XI). The maternal X-chromosome and outlines of cells with active maternal X chromosome are in bold. **a:** Random X-inactivation in the singleton zygote. **b:** XI does not occur immediately on syngamy; the result would be total non-random XI in the singleton and in the same or opposite directions in MZ twins; this degree of non-random XI has never been observed with structurally normal X chromosomes. **c:** XI might precede and trigger MZ twinning by a process of "cloning and repulsion"; the result would be reciprocal non-random XI, which has been observed in some cases (see Table III). **d,e:** Extrinsic splitting of the zygote after (and independent of) XI could result in random and non-random XI if the allocation of blastomeres were unequal. This could explain difference in patch sizes that have been observed in MZ female twins discordant for expression of X-linked genetic diseases.

[1990] reported MZ twins discordant for Aicardi syndrome. Since this is an X-linked dominant disease, non-random X-inactivation rather than mutation could explain the discordance.

MZ twins may be discordant for severity of expression of tuberous sclerosis. Gomez et al. [1982] reported on two pairs of male MZ twins with discordance for timing of onset and extent of multisystem involvement by tuberous sclerosis. A female pair of MZ twins [Kondo et al., 1991] showed, at age 20 years, marked differences in severity of tuberous sclerosis expression. Northrup et al. [1990] reported 7-year-old MZ male twins, one of whom had tuberous sclerosis while the other was phenotypically uninvolved. There is some indication of somatic mosaicism in the affected twin. In a pair of MZ twins described by Brilliant et al. [1990], high-resolution Southern blotting showed a single discordant hybridizing fragment. Again, this might be explained by a post-zygotic mutational event in one twin of this discordant pair.

Van Thienen and Van der Auwera [1994] reported a pair of twins who were proven MZ by DNA RFLPs and who were discordant for spondylocostal dysostosis, presumably on the basis of a post-zygotic mutation in one of the twins after separation of the two embryonic masses.

4. Post-Zygotic Recessive Gene Mutation

Walker [1950] reported cleft palate in one MZ twin and sporadic retinoblastoma in the co-twin. There was more marked fetal growth retardation in the twin with retinoblastoma.

5. Monozygotic Twins and Imprintable Genes

Fourteen pairs of MZ twins have been noted to be discordant for Wiedemann-Beckwith syndrome [Francheschini et al., 1993; Leonard and Johnson, 1993; Leonard et al., 1995, this volume]. The two pairs most recently reported were male, while the other twelve pairs were female. Two pairs of MZ twins have been found to be concordant for Wiedemann-Beckwith syndrome; one pair was female and the other of unspecified sex. In general, the affected twin has been heavier at birth, and there is incremental post-natal growth discordance. Although imprinting was invoked to explain the excess of discordantly affected female twins [Lubinsky and Hall, 1991], reports of discordant MZ male twin pairs reopen the question of etiology. Placentation is not known in all cases; of the two pairs reported by Leonard et al. [1996], one was monochorionic and one was dichorionic. It seems likely that the discordance might trigger MZ twinning. The relative risk of neoplastic complications in affected MZ twins is not known in comparison to singleton cases of Wiedemann-Beckwith syndrome.

MZ TWINS DISCORDANT FOR LATERAL ASYMMETRY (MIRROR-IMAGING)

This matter is confusing because there are clearly different degrees of asymmetry of viscera, varying from situs inversus totalis to right- and non-right-handedness. There does not appear to be an excess of situs inversus among MZ twins, nor of MZ twins among cases of situs inversus [Torgersen, 1950], although individual cases of discordance for situs inversus have been reported in MZ twins [Teeuw and Kok, 1992]. Handedness is complicated by the increased frequency of non-right handedness in twinning families [Bocklage, 1987]. Nevertheless, discordance for handedness is well known in MZ twins [Gedda et al., 1981]. In a detailed report, Lohr and Bracha [1992] describe MZ twins who were discordant for psychiatric illnesses. One twin was schizophrenic and the other had an affective disorder. CT scans showed mirror image differences in cerebral occipital lobe morphology. The schizophrenic twin was fully right handed while the co-twin was left handed. Dermatoglyphics were interpreted as showing greater similarity between the right hands of the twins and the left hands of the co-twins; however, this author finds the similarity of the right hand to right hand and left to left just as great. Meshkova [1992] could find no EEG evidence of mirror-imaging in MZ twins.

Viscero-atrial status, including isomerism (heterotaxy) and normal and abnormal cardiac looping, are important components of congenital heart disease; congenital heart disease is more prevalent in MZ twins than in DZ twins or singletons [Burn, 1991]. Based on small numbers, there is an association between right-sided occipital hair whorl, non-right-handedness, and heterotaxy (discordant MZ twin pairs) [Burn, 1991]. Conjoined twins offer the opportunity to consider disturbances of axis formation. In particular cephalothoracopagus (janiceps) and dicephalic conjoined twins have severely abnormal axis determination, with corresponding congenital heart disease [Machin, 1993; Machin et al., 1993; Seo et al., 1985; Girlis et al., 1993].

As mentioned previously, abnormal re-establishment of left-right asymmetry might be expected to occur in MZ twins, but the situation remains unclear at present. Detailed studies are still required, including placentation as an index of timing.

DISCORDANCE FOR MAJOR MALFORMATIONS (ESPECIALLY OF THE MIDLINE) IN MZ TWINS

Congenital anomalies in MZ twins include conjoined twinning, twin reversed arterial perfusion, birth weight discordance secondary to monochorionic placentation, other vascular complications of monochorionic placentation, and mechanical deformation because of multiple adjacent fetuses [Fogel et al., 1965]. Putting these "special and limited" anomalies aside, there is evidence that true malformations (that also occur in singletons and DZ twins) are more prevalent in MZ twins.

The original paper of Schinzel et al. [1979] is compromised by its inclusion of many like-sexed twin pairs of unproven zygosity. Discordance for major malformation is rare even in the largest series of MZ twin pairs [Cameron et al., 1983; Myrianthopoulos, 1990]. However, there are some single reports in the literature to support the idea that discordance for neural tube defect, holoprosencephaly, tracheo-esophageal fistula, vertebral anomalies, anal atresia, fistula with esophageal atresia, radial and renal dysplasia (VATER) association, ventral body wall defects, cloacal dysgenesis, and symmelia do occur in probable and proven MZ twin pairs. It has to be said that concordance for major and multiple (especially midline) malformations is also reported in MZ twins. As with all MZ twin studies, it is difficult to reach valid conclusions about prevalence and chorionicity, since chorionicity and zygosity is more often assumed than proven. Thus, the literature remains unclear, and more rigorous criteria for proof of MZ status are needed in many case reports.

Of particular interest is the high prevalence of symmelia among like-sexed twins, some of whom are documented as MZ by monochorionic placentation or multiple antigen studies. However, many are of unknown zygosity. There appears to be a definite overlap between symmelia and twin reversed arterial perfusion, as documented by Stocker and Heifetz [1987] and reported, for instance, by Hendry and Kohler [1956]. It is interesting to note that monochorionic twin pairs who are discordant for symmelia and/or bilateral renal agenesis may be monoamniotic. In these pairs, the affected twin is spared the usual deformational consequences of oligohydramnios because the unaffected co-twin adequately fills the amniotic sac with fetal urine [Davies et al.,

1971; Heydanus et al., 1993; Kohler, 1972; Maurer et al., 1974; Betti and Traisman, 1971; Johnstone and Benirschke, 1976; Marras et al., 1983]. In the case reported by Maurer et al. [1974], one monoamniotic twin had bilateral renal agenesis without deformation, while the co-twin had unilateral renal agenesis with adequate fetal urine production.

The position is less clear for holoprosencephaly. In the case report of Burck et al. [1981], a twin pair were MZ by multiple antigen testing. Both had holoprosencephaly but of varying cerebral and facial severity. Twin A had definite lateral ventricles and persistent premaxillary anlage, whereas Twin B had alobar holoprosencephaly with median cleft lip and palate. Sperber and Machin [1987] reported monochorionic MZ fetuses dying of twin transfusion syndrome who were discordant for holoprosencephaly. Several other cases of like-sexed twin discordance for holoprosencephaly are reported, but MZ status is assumed rather than proven.

There are several reports of discordant MZ twins with oculoauriculovertebral dysplasia [Goldenhar complex), summarized by Boles et al. [1987]. Of 5 cases fulfilling criteria for MZ twinning, placentation was known to be monochorionic in 3 pairs and dichorionic in 2 pairs. Thus, vasculogenic mechanisms cannot satisfactorily explain all MZ cases. An early disturbance of cephalogenesis clearly occurs, and this may be provoked by the twinning event itself. Affected MZ twins are of lower birth weights than their co-twins [Boles et al., 1987].

It has been suggested that discordance in MZ twin pairs for amyoplasia [Hall et al., 1983] and agangliono-sis [Siplovich et al., 1983] may be caused by microvascular events in early embryogenesis in the context of monochorionic placentation. However, all the general causes of maldevelopment (e.g., unequal allocation of blastomeres) could apply equally well.

Major abnormal blastogenesis may account for the frequently reported occurrence of discordance in MZ twins for lateral body wall defect and other forms of "amniotic band syndrome." Close apposition of the developing axes of the MZ twins could interfere with closure of the connections of intra- and extra-embryonic coelom. Illustrative cases were published by Fiedler and Phelan [1983], Herva and Karkinen-Jaaskelainen [1984], Kancherla et al. [1981], Khudr and Benirschke [1972], Worthen et al. [198], and Pysher [1980].

MZ twin studies of cerebral ventricular size show high concordance rates in comparison with DZ twins. However, in MZ twins discordant for schizophrenia, there is also discordant cerebral ventricular size within twin pairs [Reveley et al., 1982].

DISCORDANT FETAL GROWTH IN MZ MONOCHORIONIC TWINS—CONSIDERATIONS OF PLACENTAL VASCULAR ANATOMY

Little is known about mechanisms by which the autonomous fetal and placental circulations become linked. However, the monochorionic twin placenta is truly a single organ, to which at least two fetal circulations become linked in multiple monochorionic pregnancy. The two or more fetal umbilical arterial and venous systems may be connected to equal or unequal zones of placental perfusion, quite apart from any considerations of superficial placental vascular anastomoses. Thus, it is possible for various combinations of vascular perfusion zones to occur, as listed in Table III and diagrammed in Figure 4 [Machin et al., 1996].

In the absence of superficial arterio-arterial and/or veno-venous anastomoses, type 1 vascularity is likely to cause significant fetal growth discordance without twin-twin transfusion, type 2 will result in growth-concordant twins who are likely to go to term without complications, while types 3 and 4 are likely to result in prenatal twin-twin transfusion. This is because part of the arterial perfusion of the one twin (donor) returns to the larger, overlapping venous return zone of the other twin (recipient) [Bendon and Siddiqui, 1989]. The presence of arterio-arterial and veno-venous anastomoses further complicates these issues, and explains why the prenatal diagnostic criteria for twin-twin transfusion are uncertain [Danskin and Neilson, 1989; Wenstrom et al., 1992]. In general, arterio-arterial and veno-venous anastomoses act as direct inter-twin connections that can actually mitigate the effects of potential twin-twin transfusion (see accompanying paper).

In twin reversed arterial perfusion, there are large arterio-arterial and veno-venous anastomoses, and the acardiac twin receives perfusion almost entirely from the pump co-twin rather than from any part of the monochorionic placenta. Twin reversed arterial perfusion may result from major cardiac malformation in the acardiac twin, close insertions of the two cords with early

TABLE III. Some Patterns of Zonal Allocation of Arterial and Venous Perfusion in Monochorionic Twin Placentas

	Twin A	Twin B	Result
1	Larger arterial zone ↓ larger venous zone	Smaller arterial zone ↓ smaller venous zone	B growth retarded
2	Normal (equal) arterial zone ↓ normal (equal) venous zone	Smaller arterial zone ↓ normal or larger venous zone	Equal fetal growth, uncomplicated MC pregnancy
3	Larger arterial zone ↓ normal or smaller venous zone	Smaller arterial zone ↓ normal or larger venous zone	TTT, A donor, B recipient
4	Normal arterial zone ↓ larger venous zone	Normal arterial zone ↓ smaller venous zone	TTT, A recipient, B donor

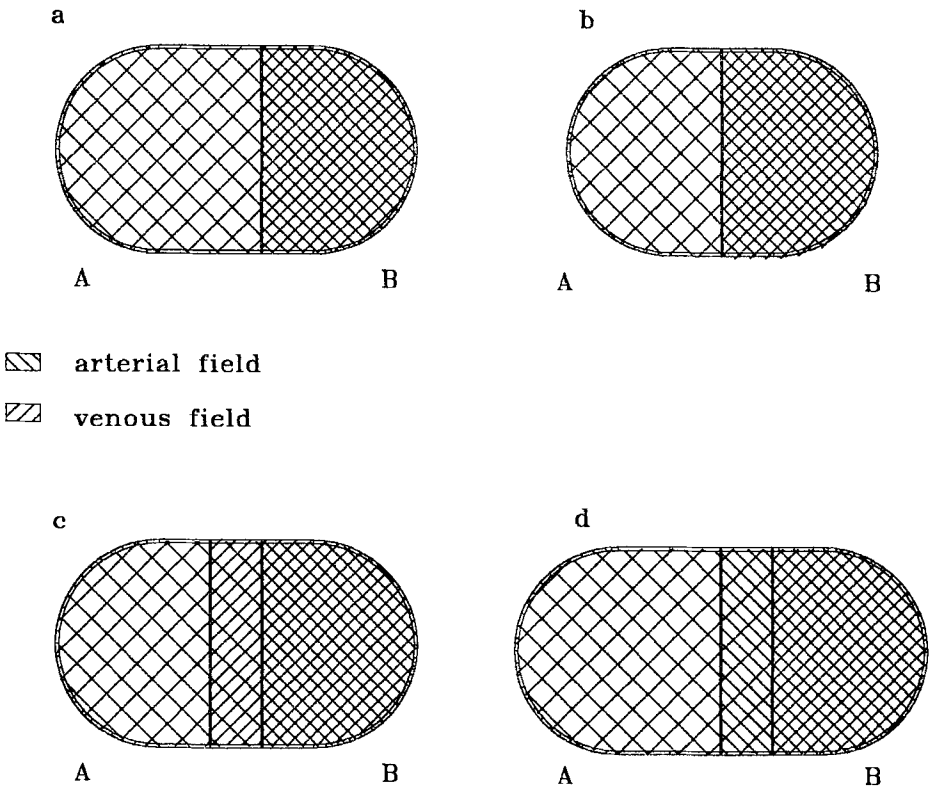

Fig. 4. Arterial and venous zonal patterns in MC twin placentas and their implications for fetal growth and twin transfusion. **a:** Type 1. **b:** Type 2. **c:** Type 3. **d:** Type 4 as per Table IV.

"dominance" of the two umbilical circulations by the pump twin, or from failure of connection between the somatic and placental parenchymal circulations of the acardiac twin. The pump twin perfuses the acardiac fetus by retrograde flow in the umbilical vessels of the acardiac twin [Kirkinen et al., 1989]. A significant number of acardiac fetuses are chromosomally abnormal, with confined MZ twin mosaicism [reviewed by Wolf et al. [1991]. In these cases, cardiac malformation may cause cardiac failure and compensatory assumption of perfusional function by the pump twin. In the absence of superficial anastomoses, a vanishing fetus or fetus papyraceus would result. Symmelia and twin reversed arterial perfusion frequently co-exist [Stocker and Heifetz, 1987].

VANISHING TWINS AND FETUS PAPYRACEUS

There is an excess of monochorionic twin pairs among spontaneous abortuses [Livingstone and Poland, 1980; Uchida et al., 1983; Benirschke, 1961]. Intrauterine death of one twin fetus can occur at any gestational age in spontaneous multiple pregnancies. When fetal demise occurs early, often in the first trimester, a "vanishing twin" may result. There is no information on the proportion of vanishing twins that originated as MZ twins, although any of the conditions causing MZ twin discordance could cause vanishing twins.

Fetus papyraceus refers to compressed or flattened recognizable fetal remnants from fetal demise in the second trimester. The effect on the surviving co-twin presumably depends largely but not entirely on zygosity and chorionicity. In the large series analyzed by Kindred [1944], 141 cases had information about chorionicity; 93 cases (66%) were DC, whereas 48 (34%) were monochorionic, predominantly monochorionic diamniotic. There is a particular association between fetus papyraceus and the presence in the monochorionic cotwin of aplasia cutis and bowel atresia [Wagner et al., 1990]. These anomalies are probably caused by acute hemodynamic changes in monochorionic vascular arrangements at the time of the death of one fetus. Nance [1981] has reported resolution of hydrops and hydramnios in the recipient of twin-twin transfusion following death and mummification of the donor twin.

Fetal death of one monochorionic twin in the third trimester (often secondary to twin-twin transfusion or twin reversed arterial perfusion) produces a different constellation of lesions in the survivor. Cerebral, renal, splenic, hepatic, pulmonary, and myocardial infarctions have been reported [Benirschke, 1961; Patten et al., 1989; Yoshida and Soma, 1986; Enbom, 1985]. Similar lesions may result from attempts at selective terminations of monochorionic pregnancies in which the circulation of the intended survivor is not adequately protected [Golbus et al., 1988; Donnenfeld et al., 1989].

PSEUDO-CONCORDANCE IN MZ TWINS

Reference has already been made to the concordant absence of oligohydramnios syndrome in MZ, mono-

chorionic, monoamniotic twins, only one of whom has fetal anuria [Kohler, 1972; Maurer et al., 1974; Betti and Traisman, 1971; Marras et al., 1983]. Discordance for congenital hypothyroidism in monochorionic twins was masked, at neonatal screening, by prenatal transfusion of thyroid hormones from the normal co-twin via placental anastomoses [de Zegher and Vanderschueren-Lodeweyckx, 1989]. In the case of monochorionic twins, fetal death or a-fetoprotein-producing anomaly of one fetus produces high amniotic a-fetoprotein levels in the sac of the co-twin [Stirrat et al., 1979; Winsor et al., 1987; Holbrook et al., 1987]; this is because a-fetoprotein can diffuse across a septum consisting of two amniotic layers only, but cannot do so when there is also intervening chorion, as in dichorionic twinning. Such "induced" high a-fetoprotein levels in the amniotic sacs of anatomically normal monochorionic co-twins have led to termination of both fetuses.

SUMMARY AND CRITIQUE

There is at present a destabilizing influence on twin studies. This is the recognition that there are many reasons why MZ twin pairs may be phenotypically and genotypically discordant. This paper has reviewed a number of prenatal environmental and genetic causes for such discordance. This realization throws in serious doubt any facile assumptions about "nurture and nature" in DZ and MZ twins; it clearly indicates that many MZ twins are not "identical"; but it also demonstrates that such discordance has much information to offer about mechanisms of MZ twinning and of early development of normal and abnormal singleton embryos.

While most MZ twins are remarkably similar, the unusual exceptions are intellectually stimulating and also a challenge to clinical management.

A MODEST PROPOSAL FOR MULTICENTRE TWIN STUDIES

As has been mentioned at several points in this paper, many classic publications about MZ twins and twinning are, in fact, lacking in the appropriate criteria for the diagnosis of MZ status. Like-sexed twins and the use of the Hardy-Weinberg law are no substitute for definitive diagnosis of MZ twinning.

In general, diagnosis of MZ twinning is simple. Monochorionic twins are MZ; like-sex twins can be tested for DNA restriction fragment length polymorphism differences, using placenta, cord, and membranes.

In order to discover the frequency and significance of discordant events in MZ twinning, it seems to this author that these minimal criteria for MZ status could be applied on a multi-centre basis for the organization of a multidisciplinary study of a large cohort of MZ twins. In this way, significant numbers of MZ twins, concordant and discordant for phenotypic and genotypic traits, could be followed from birth. Simple issues (such as the prognosis for growth catchup after birth weight discordance of various causes) could be studied in numbers not available from single centers.

The establishment of zygosity at birth not only answers the question most frequently asked by parents and relatives; it is also an issue of the "right to know," and has medical and educational consequences [Derom et al., 1991]. Among the medical consequences are the access to rejection-free transplanted organs, such as skin for burns [Westerveld et al., 1986], closure of neural tube defect [Barwick et al., 1993], and hypospadias repair [Donovan and Maizels, 1986], and parathyroid gland for post-thyroidectomy permanent hypoparathyroidism [Segerberg et al., 1992], renal transplantation [Tilney, 1986], testis transplantation [Silber, 1978], and bone marrow transplantation [Champlin et al., 1984]. There would be great benefits to genetic and, particularly, twin research if the simple matter of zygosity was adequately diagnosed at or before birth.

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